



A comprehensive phylogeny of the genus *Kurixalus* (Rhacophoridae, Anura) sheds light on the geographical range evolution of frilled swamp treefrogs

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ABSTRACT

Currently, the genus *Kurixalus* comprises 14 species distributed in Southern, Southeast and East Asia. Because of their relatively low dispersal capability and intolerance of seawater, this group is ideal for the study of terrestrial range evolution, especially that portion of its range that extends into the island archipelagos of Southern Asia.

We assembled a large dataset of mitochondrial and nuclear genes, and estimated phylogeny by maximum likelihood and Bayesian methods, and we explored the history of each species via divergence-time estimation based on fossil-calibrations. A variety of ancestral-area reconstruction strategies were employed to estimate past changes of the species' geographical range, and to evaluate the impact of different abiotic barriers on range evolution. We found that frilled swamp treefrogs probably originated in Taiwan or South Vietnam in the Oligocene. Alternatively, the lineage leading to *Kurixalus appendiculatus* strongly supports a hypothesis of terrestrial connection between the Indian and Asian continents in the Oligocene. The outcome of both our divergence-time estimates and ancestral-area reconstruction suggests that the divergence between species from Indochina and Taiwan can probably be attributed to the opening of the South China Sea, approximately 33 million years ago. We could not find evidence for dispersal between mainland China and Taiwan Island. Formation of both Mekong and Red River valleys did not have any impact on *Kurixalus* species diversification. However, coincidence in timing of climate change and availability of plausible dispersal routes from the Oligocene to the middle Miocene, plausibly implied that *Kurixalus* diversification in Asia resulted from contemporaneous, climate-induced environmental upheaval (Late Oligocene Warming at 29 Ma; Mi-1 glaciation since 24.4–21.5 Ma; Mid-Miocene Climatic Optimum at 14 Ma), which alternatively opened and closed dispersal routes.

1. Introduction

The range of the genus *Kurixalus*, a member of the family Rhacophoridae, encompasses large parts of East and Southeast Asia, including the Himalayan front ranges (eastern India through Myanmar and mountainous southern China), south to southern Cambodia, Laos and central Vietnam, through western and northern peninsular Thailand to Malaya to Sumatra, Borneo, Taiwan, the Ryuku islands, and the Philippines (Frost, 2016). Members of this genus generally are delicate arboreal tree frogs, which breed exclusively in shallow freshwater swamps. Anurans in general possess extremely weak saltwater

tolerance (Hopkins and Brodie, 2015; Zug et al., 2001), and these physiological limitations imply that range expansion across ocean barriers may be unlikely. Despite several recent studies demonstrating that amphibians do colonize oceanic islands by rafting on debris discharged from freshwater river systems (Bell et al., 2015; De Queiroz, 2014), the genus may potentially be a good biogeographical model system, with its natural history and distribution likely reflecting large-scale geological and climatic changes in the complex East and Southeast Asian landscape (Che et al., 2010; Li et al., 2013).

Although recent studies successively made valuable taxonomic contributions (Frost et al., 2006; Hertwig et al., 2013; Li et al., 2008,

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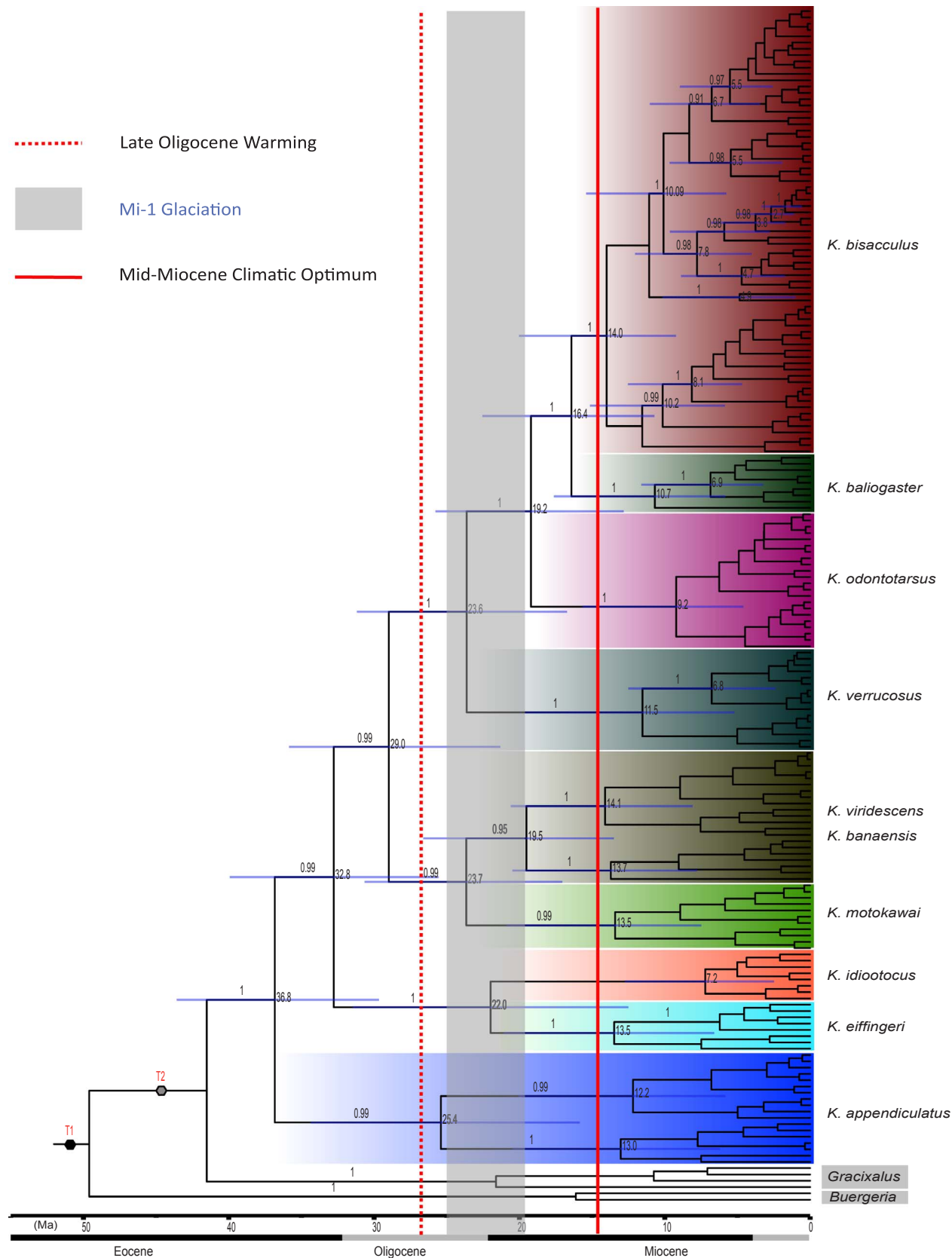


Fig. 1. Divergence-time estimates of the *Kurixalus* species based on Dataset 1 (mitochondrial DNA). The climatic hypothesis included Late Oligocene Warming, Mi-1 Glaciation and Mid-Miocene Climatic Optimum as described by Zachos et al. (2001). T1 and T2 were the two calibration points in the phylogenetic tree.

2009; Nguyen et al., 2014a, 2014b; Rowley et al., 2011; Yu et al., 2010, 2013), a complete phylogeny for the genus is still unavailable. However, recent accumulation of molecular sequence data provides an opportunity for a comprehensive phylogenetic analysis of *Kurixalus*. We undertook the present study not only to guide systematic treatments on the level of genus, but also to estimate time-calibrated divergence and the evolution of distributional range, in an attempt to understand range evolution through time and its possible correlation with abiotic changes to the geographical template. With this approach we sought to address whether speciation was influenced by vicariance or dispersal events over marine barriers. We used currently available molecular sequence data to evaluate two basic questions in *Kurixalus* natural history: (1) What are the phylogenetic relationships of species in the genus *Kurixalus* and (2) do phylogenetic patterns coincide spatially and temporally with changes in East/Southeast Asian landscape or climate?

2. Materials and methods

2.1. Data preparation

We downloaded, from NCBI Genbank (National Center for Biotechnology Information) (see Supplement Table 1), sequence data that originated from previous analyses (Frost et al., 2006; Hertwig et al., 2013; Li et al., 2008, 2009, 2013; Nguyen et al., 2014a, 2014b; Rowley et al., 2011; Yu et al., 2010, 2013). Three mitochondrial genes and five nuclear genes were surveyed: partial 12S rRNA, tRNA-Val, and 16S rRNA genes were regarded as a single mitochondrial locus; the other five nuclear loci included (i) a fragment of the brain-derived neurotrophic factor (*BDNF*); (ii) a fragment of proopiomelanocortin (*POMC*); (iii) a fragment of the recombination activating gene 1 (*RAG-1*); (iv) a fragment of exon 1 of rhodopsin (*RHOD*), and (v) a fragment of exon 1 of tyrosinase (*TYR*). Mitochondrial 12S rRNA, tRNA-Val, and 16S rRNA sequences of *Kurixalus eiffingeri* from Ishigaki Island (Ryukyu Islands) (see the last one in Supplement Table 1 for detailed information) were newly acquired according to protocols of previous studies (Li et al., 2008, 2009).

Sequences from mitochondrial DNA and five nuclear loci were aligned with MUSCLE 3.8.31 (Edgar, 2004) with 16 iterations and gap open and extension penalties of -400 and 0 , the alignment was checked again with MAFFT version 6 (Katoh et al., 2002), and we compared both resulting alignments and adjusted inconsistencies manually. When we assembled the data, we firstly checked and adjusted the order of mitochondrial genes and nuclear genes to assure that different genes come from one individual according to original source studies. In cases where only mitochondrial data were available for a species, we treated nuclear partitions for these individuals as missing data. In summary, we assembled two data matrices: one consisting of just mitochondrial gene fragments (Dataset 1), and another in which we combined both mitochondrial and nuclear genes (Dataset 2). The two datasets were compared against one another in cases of topological inconsistency in phylogeny estimation.

Saturation of each gene was tested by checking the numbers of transitions and transversions against Kimura 2-parameter distance (Kimura, 1980) using DAMBE 5.2.5 (Xia and Xie, 2001). The partition-homogeneity test as implemented in PAUP 4.10beta (Swofford, 2002) was used to examine whether different gene loci could be concatenated ($P > 0.05$).

2.2. Phylogenetic analyses and divergence-time estimation

Our divergence-time estimate was solely based on mitochondrial data, but we used our second dataset (mitochondrial and nuclear data) to verify our inferred topology. We inferred divergence time using a Bayesian Metropolis coupled Markov chain Monte Carlo approach in BEAST MC3 1.7.5 (Drummond and Rambaut, 2007) with three chains ($\delta = 1.0$). The preferred nucleotide substitution model was

identified in jModelTest 2.1.5 (Posada, 2008) under the Akaike Information Criterion (AIC). After a preliminary series of test runs, we used an uncorrelated lognormal relaxed molecular-clock model and a Yule tree prior. The Markov chain Monte Carlo (MCMC) was run for 50 million generations and sampled every 1000 generations. Convergence of MCMC chains and effective sampling size were checked in Tracer 1.5 (Drummond and Rambaut, 2007), ensuring a sample size > 200 for all parameters.

We calibrated the age of the most recent common ancestor (MRCA) of the family Rhacophoridae with the age of fossil *Indorana prasadi* from Early Eocene sediments of India (Folie et al., 2013). The prior distribution for a fossil-based age constraint was modelled with a Γ -distribution (shape value = 4; scale factor = 2) with the fossil's minimum age of 45 Ma (T1 in Fig. 1) as offset (5–95% interquartile range 47.5–60.5 Ma). Additionally, we implemented a secondary calibration point (T2 in Fig. 1) from the study of Li et al. (2013) for the divergence point between the genus *Kurixalus* and *Gracixalus*; implemented as a normally-distributed calibration density (mean = 38.3; stdev = 2.5; 5–95% interquartile range 32.54–44.06 Ma).

To confirm the topology found in the divergence-time analysis, we also conducted Maximum Likelihood (ML) and Bayesian inference (BI) analyses for Dataset 2. ML was conducted with RAXML 8.0.17 (Stamatakis, 2006) using a GTR+I+ Γ model for our final likelihood search, and switched to the per-site rate category model during fast bootstrapping with 1000 replicates. The alignment was partitioned for each locus. Bayesian phylogenetic inference was conducted in MrBayes 3.2.2 (Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012) with substitution models for each partition as inferred by jModelTest based on the AIC. We conducted two parallel runs for 5 M generations with three chains each, sampling every 1000th generation and discarding the first 20% of samples as burn-in (stationarity and effective sampling size were checked in Tracer; average standard deviation of split frequencies = 0.008), and calculated the maximum clade credibility tree in TreeAnnotator 1.7.5.

2.3. Ancestral area estimation

We compiled distributional data for each *Kurixalus* species from original studies and assigned the taxa to geographical ranges as defined below. To evaluate the influence of different types of barriers on range evolution, we estimated ancestral ranges for two different range assignments (RA) (“RA-1” and “RA-2”). “RA-1” focused on the insular range of *Kurixalus* with five geographical areas included (Fig. 4a): mainland Asia (area A); Sundaland (area B); the Philippines (area C); Taiwan (area D) and the Ryukyu Islands (area E). We focused this round of analyses on these five biogeographical areas, which are separated by oceanic channels or biogeographic barriers, because part of our goal was to elucidate possible occurrences of historical dispersal across ocean barriers, followed by subsequent landmass colonization. At the completion of this initial round of analyses (“RA-1”), we found that speciation of *Kurixalus* species was concentrated in more distal portions of our estimated topology (note branching order of lineages leading to *K. motokawai* and *K. bisacculus*; Fig. 1). In our second round of analyses (“RA-2”), which we designed to consider factors possibly influencing speciation, we conducted analyses focusing on continental barriers among five areas (elevational boundaries and the course of the Mekong and Red River; Fig. 4b). We identified five general areas consisting mainly of: northern Indochina (area A₁); Southern China (area A₂); main Myanmar (area A₃); main Thailand (area A₄), and southern Indochina (A₅). The justification for the designation of these areas was based on Bain and Hurley (2001), in which sharp changes in elevation and river courses were found to influence the faunal constitution in Indochina (including Cambodia, Laos and Vietnam). Biogeographic inferences were obtained by applying statistical dispersal-vicariance analysis (S-DIVA) implemented in RASP 3.1 (Yan et al., 2015) using the following settings: two thousand randomly selected post burn-in BEAST

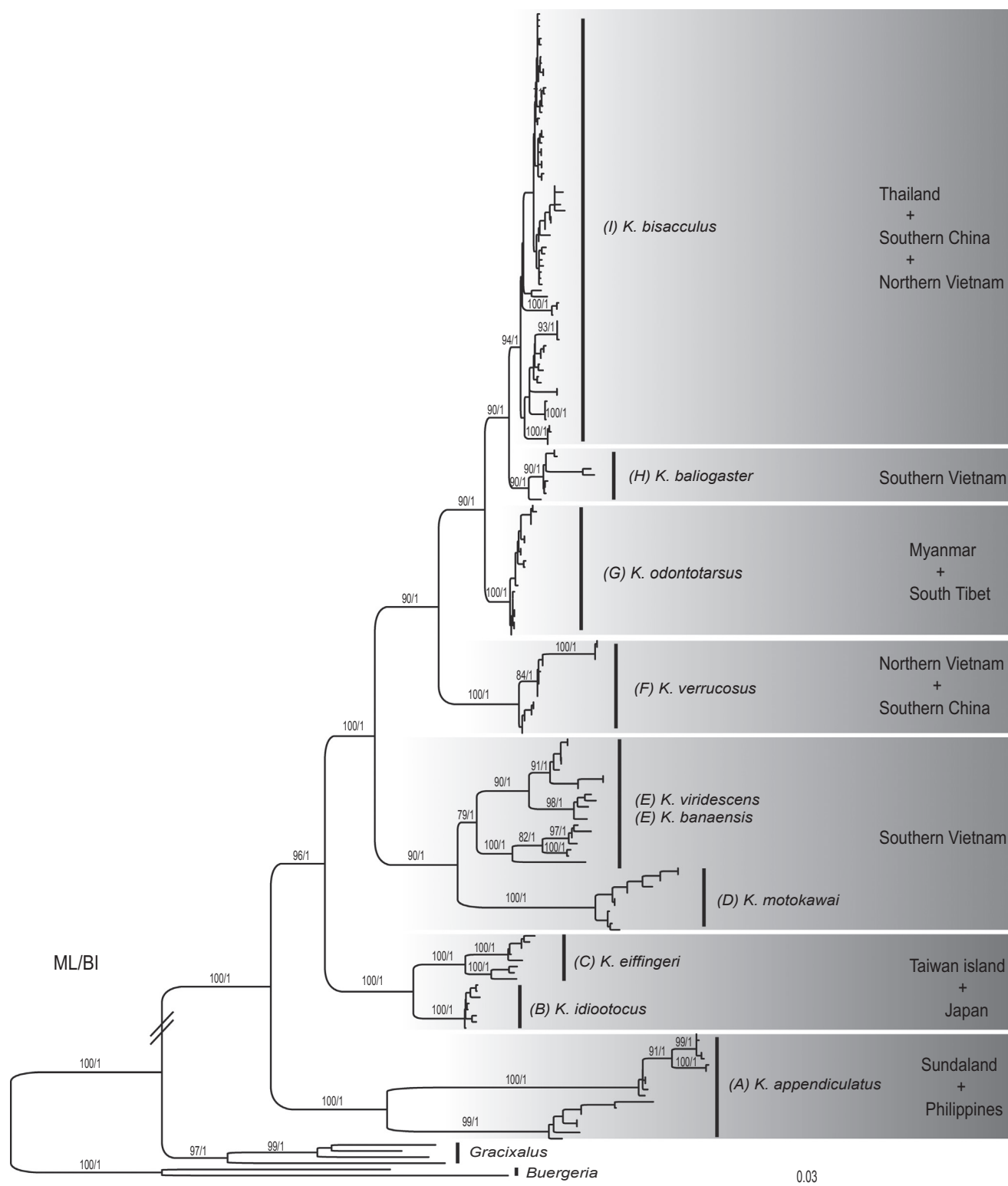


Fig. 2. Phylogenetic tree inferred from mitochondrial and nuclear DNA (maximum clade credibility tree of the Bayesian phylogenetic inference; with bootstrap values of the ML and posterior probabilities of the BI analyses). The bar provides the scales of evolutionary lineages changing over time; thus, the longer the branches, the larger the amount of change.

trees were used, and the maximum number of individual unit areas was set to two. In addition to these event-based biogeographic methods, we also applied a parametric approach based on the dispersal, extinction, and cladogenesis model (DEC) (Ree and Smith, 2008) using the source code of C++ version of Lagrange as implemented in RASP. The analysis used the maximum clade credibility tree of the Bayesian phylogenetic analysis, and the same area-coding as for the event-based

methods.

Simultaneously, we pruned a species-level tree from the BEAST maximum clade credibility tree using the R-package “ape” (Paradis et al., 2004). Although samples used in the analysis of RASP covered a part of the geographic range of the genus *Kurixalus*, based on the record of Frost (2016), several unreliable locations, for which *Kurixalus* species have been reported (older unsubstantiated literature records), were

removed and not included in subsequent analyses. In addition, we note some controversy with regards to doubtful records being included (or not) in the distributional range of the species *Kurixalus appendiculatus*. Therefore, to insure accuracy of our analyses, we additionally designed two distributional matrices: (1) the first matrix (Supplemental Table 2) is based on Frost (2016); while (2) the second matrix (Supplemental Table 3) excludes dubious reports of *K. appendiculatus* from southern mainland Asia (Vietnam, Myanmar, and Thailand). We suspect that these older records likely correspond to populations now assigned to different species. Ancestral-area estimation was conducted with the R package “BioGeoBEARS” (Matzke, 2013), to distinguish between different biogeographical models: The Dispersal Extinction Cladogenesis model (DEC, DEC+J) and the Likelihood equivalent of the Dispersal-Vicariance approach (DIVALIKE, DIVALIKE+J). We conducted these analyses both with, and without, the J parameter, which allows for colonization of an area not occupied by parental lineages (e.g., long-distance dispersal to an isolated landmass). The DEC model is identical to the Lagrange DEC model, but ancestral-state probabilities at nodes are slightly different, because BioGeoBEARS reports the ancestral-state probabilities under the global ML model, whereas Lagrange reports ancestral-state probabilities by re-optimizing the likelihood after fixing the state at each node (Matzke, 2014). The BioGeoBEARS “DIVALIKE” model is not identical with Ronquist’s (1997) parsimony DIVA. It is a likelihood interpretation of DIVA, constructed by modelling DIVA’s processes similar to DEC, but penalizing dispersal relative to vicariance. We set the maximum number of distribution areas to three, and we compared four models and selected the best under the AIC weight. Finally, we based our interpretation on a comparison of the results from the two geographic-range matrices to infer ancestral-areas across the clade.

3. Results

3.1. Data set completeness and sequence characteristics

In total, we assembled 183 non-overlapping sequences belonging to *Kurixalus*. Sequences corresponding to ten species of *Kurixalus* in our analysis were derived from previous taxonomic studies. Among all of these, at least 12S and/or 16S rRNA genes were included in our final matrix. The five nuclear genes were variably represented: (i) 18 sequences of *BDNF*; (ii) 20 of *POMC*; (iii) 30 of *RAG-1*; (iv) 30 of *RHOD*, and (v) 34 of *TYR*. We selected specimens belonging to the genera *Gracixalus* and *Buergeria* as outgroups. Details are included in Supplemental Information Table 1. After alignment and adjustment, the mitochondrial locus and five nuclear genes (i–v) produced 2512, 611, 592, 1164, 327 and 532 nucleotide positions, respectively.

The six genes were unsaturated in the separate test of substitutional saturation. Our partition-homogeneity tests demonstrated that each gene could be treated as a separate partition (locus) in subsequent analyses, while the concatenation was refused ($P > 0.05$). The appropriate nucleotide-substitution models were examined by jModelTest for our mitochondrial and five nuclear genes (i–v) were GTR+I+ Γ , GTR+I, TIM2+ Γ , HKY+I, HKY+I and SYM+ Γ , respectively.

3.2. Phylogenetic estimate and divergence-date estimation

The two phylogenetic methods (BI, ML) equally resolved topologies that were completely congruent at the species level (see Figs. 1 and 2); each major clade was strongly supported in both classes of analyses. Our phylogeny strongly supported monophyly of the genus *Kurixalus* and the most species: Clade A: *K. appendiculatus*, Clade B: *K. eiffingeri*, Clade C: *K. idiootocus*, Clade D: *K. motokawai*, Clade F: *K. verrucosus*, Clade G: *K. odontotarsus*, Clade H: *K. baliogaster*, and Clade I: *K. bisacculus*. However, Clade E contains two species that are not individually monophyletic (*K. banaensis* and *K. viridescens*). Given the two species’ morphological distinctiveness (Nguyen et al., 2014b), we make

no taxonomic changes here pending future study of this clade.

With additional calibration points (Dataset 1), the topology resulting from the phylogenetic analyses including divergence-time estimation was resolved, and branches were strongly supported (and consistent with both ML and BI trees), despite the absence of the nuclear data. The lineage leading to *Kurixalus* originated at approximately 36.8 Ma (see Fig. 1).

3.3. Ancestral-area estimation

Our BioGeoBEARS model-selection step demonstrated a clear preference for DEC (Supplemental Table 4) and DIVALIKE (Supplemental Table 5), and all ancestral-area analyses identified South Vietnam or Taiwan as the most probable original area (see Supplementary Figs. 3 and 4 and Fig. 4c and d) corresponding to the divergence of the *Kurixalus* lineage from its closest rhacophorid relatives.

Our ancestral-area estimates include the species now found in oceanic archipelagos, suggesting variation in the degree to which oceanic barriers limit geographical ranges of treefrogs. For example, the channel between insular Taiwan and surrounding areas appears as a strict barrier to faunal exchange, as implied by the divergence between *K. eiffingeri* and *K. idiootocus* (Clades C and B).

Speciation leading to *K. eiffingeri* and *K. idiootocus* (Taiwan and adjacent areas) resulted from overseas dispersal (S-DIVA and DEC models in RASP), and the Ryukyus species *K. eiffingeri* appears to have originated by vicariance, when this archipelago separated from Taiwan (Supplementary Fig. 1). When *Kurixalus* species from Indochina and China were considered (RA-2), a series of dispersal events was inferred. The initial range expansion is suggested to have occurred from southern Indochina to southeast China. Subsequent dispersal from southern China back into Indochina (Supplementary Fig. 2 and Fig. 4b) is secondarily inferred. We found only weak support for the role of the Red River and Mekong River functioning as generators of vicariance. The inferences of dispersal were identical in S-DIVA and DEC analyses.

4. Discussion

4.1. Quality of data structure

Despite a few instances of missing data and controversial locality records, causing exclusion of some samples from this study, our analysis constitutes the most comprehensive phylogenetic study of *Kurixalus* performed to date. Ten species (*K. appendiculatus*, *K. baliogaster*, *K. banaensis*, *K. bisacculus*, *K. eiffingeri*, *K. idiootocus*, *K. motokawai*, *K. odontotarsus*, *K. verrucosus* and *K. viridescens*) were included, representing about 71 percent of all *Kurixalus* species known. Newly erected species *K. beryliniris* and *K. wangi* were actually separated from previously recognized species *K. eiffingeri* (Wu et al., 2016) and, as such, the three species could likely be regarded as a monophyletic group, indicating that we have elucidated the major lineage branching patterns of *Kurixalus* phylogeny. Currently, there are no available molecular sequence data for *Kurixalus naso*. Additionally, we did not include the single available sequence of *K. ananjevae*, due to its likely belonging to the genus *Gracixalus* (Rowley et al., 2011). Although the phylogenetic position of these two species is still unknown, our phylogeny is otherwise well resolved and well supported. Finally, congruence in topologies estimated from mitochondrial versus nuclear genes argued against the presence of mitochondrial introgression that could potentially mislead our phylogenetic estimates.

4.2. Colonization of the Indian subcontinent

Recent geological reconstructions of the Indian subcontinent colliding with Asia have been the subject of many biogeographic studies, suggesting a key function of Indian continental drift in species exchange and isolation (Briggs, 2003; Karanth, 2006; van der Meijden et al.,

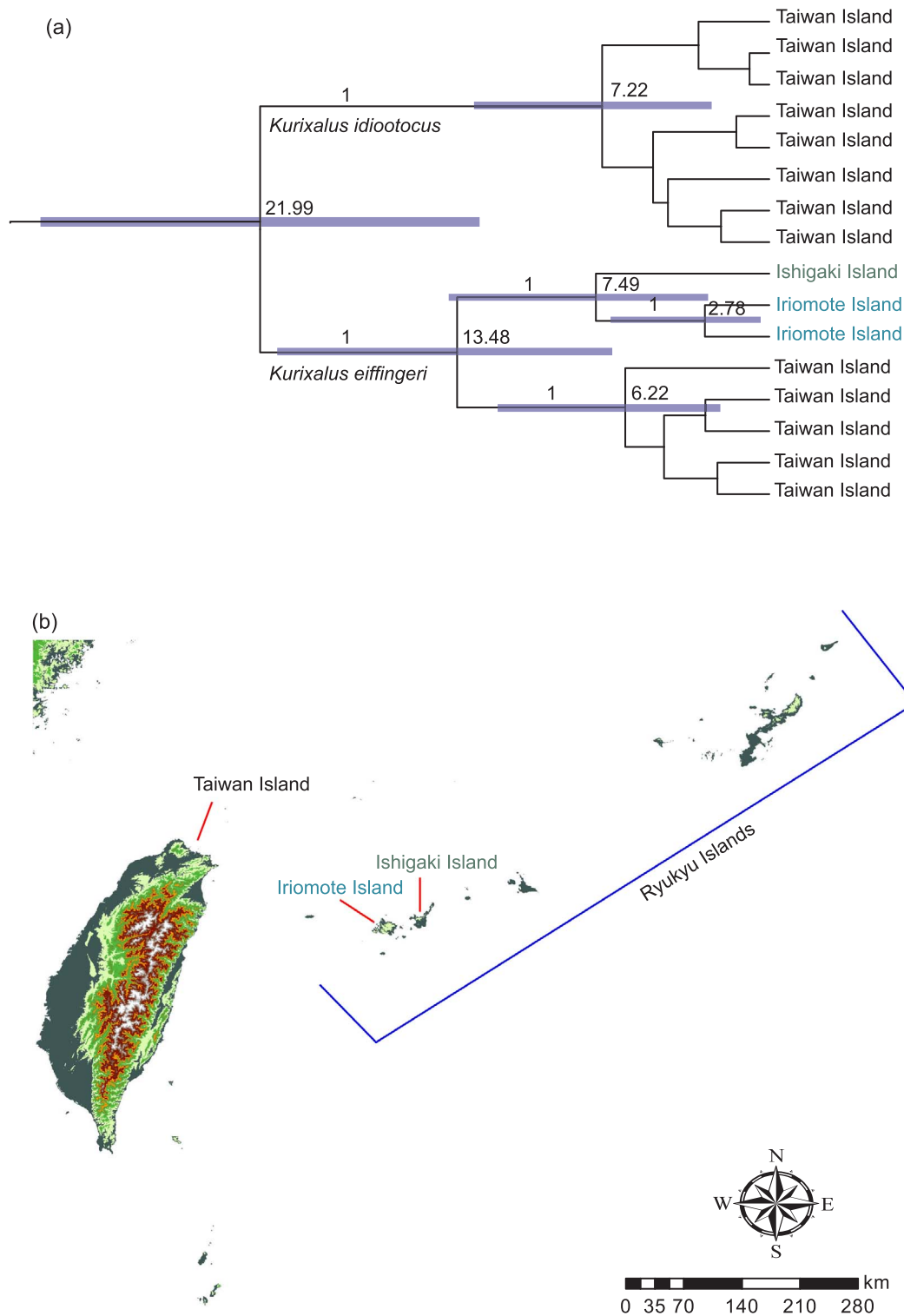


Fig. 3. The timing of speciation of the Taiwanese species of *Kurixalus*. (a) The time divergence between the sister-species *K. idiootocus* and *K. eiffingeri*, occurred at about 22 Ma, secondly, within *K. eiffingeri*, the Ryukyu Islands populations diverged to Taiwan Island (b) at about 13.5 Ma.

2007; Grismer et al., 2016; Klaus et al., 2016). However, the hypotheses suggesting faunal exchange before the actual collision with Tibet have been the subject of debates (Ali and Aitchison, 2006; Leech et al., 2005; Li et al., 2006), which have concentrated on the question of whether the Indian subcontinent had a terrestrial bridge with the Indochinese plate (Acton, 1999), or just a tectonic collision but no above-ocean land contact (Schettino and Scotese, 2005).

The most recent common ancestor of genus *Kurixalus* likely

occurred in the areas around southern Indochina and Taiwan. Dinesh et al. (2013) reported *K. appendiculatus* in India. If true, two routes may have allowed *K. appendiculatus* to disperse from Indochina into the Indian subcontinent: one hypothesis is that *K. appendiculatus* colonized the Indian subcontinent via a terrestrial land bridge, and the second interpretation is that *K. appendiculatus* colonized northern Indochina and entered India via today's Assam Region. However, records of *K. appendiculatus* from Thailand and Myanmar most likely are incorrect

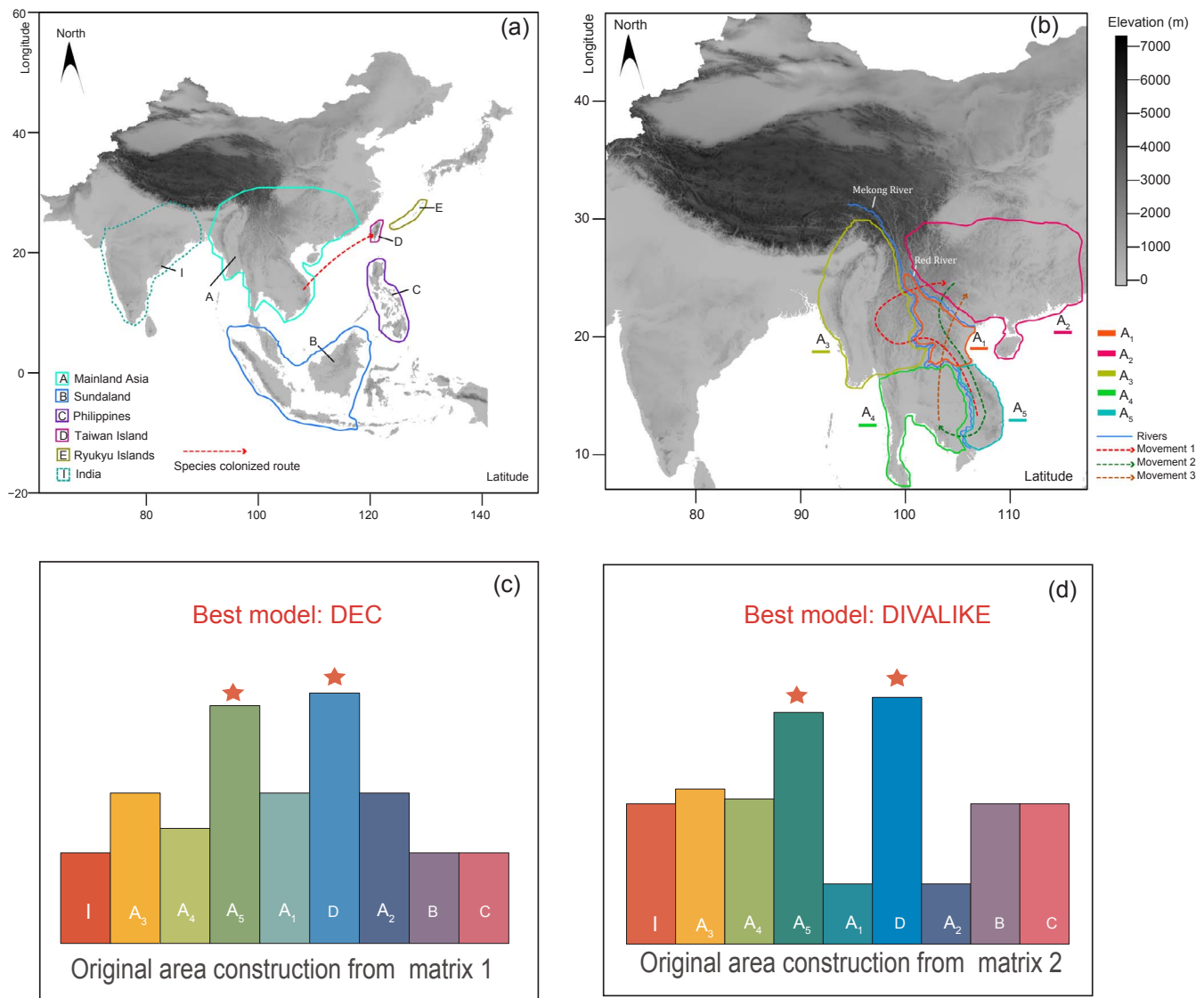


Fig. 4. Two different range assignments (RA) (“RA-1” and “RA-2”) to evaluate the influence of different types of barriers on range evolution and Original-area construction deduced from different distributional matrices of *Kurixalus*, using the best selected model of DEC and DIVALIKE. (a) “RA-1”: mainland China (area A₁); Sundaland (area B); the Philippines (area C); Taiwan (area D) and the Ryukyu Islands (area E). The red dashed arrow indicates the higher possibility of Taiwan species of *Kurixalus* derived from Southeastern China in the ancestral area estimation. (b) “RA-2”: northern Indochina (area A₁); Southern China (area A₂); main Myanmar (area A₃); main Thailand (area A₄), and southern Indochina (A₅). With higher probability Asian *Kurixalus* species initially dispersed northwards into China between 29.0 and 23.6 Ma (Movement 1), followed by back-dispersal in a southwards direction until 19.2 Ma (Movement 2), and subsequently colonized the north again (Movement 3). (c) and (d): The best selected model of DEC and DIVALIKE from different distributional matrices of *Kurixalus*, which suggesting the southern Indochina and Taiwan (represented by red star) appear likely to be the origin region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

identifications of *K. bisacculus* (Nguyen et al., 2014b), because *K. appendiculatus* does not occupy most parts of Indochina. Therefore, the approximate range of this species should be restricted to current Sundaland, Philippines and India. Thus, our results tend to support the first geological interpretation based on the trajectory of the Indian subcontinent, with the latter not only being close to the Indochinese plate during its northwards drift, but also having formed a terrestrial corridor allowing for terrestrial faunal exchange about 37 million years ago. This interpretation has initially been articulated for freshwater crabs (Klaus et al., 2010) and was later also confirmed for Indian Dragon lizards (Grismer et al., 2016), which lend support to our similar interpretation for treefrogs.

4.3. Taiwan species formation

The species of *K. eiffingeri* and *K. idiotocus*, potentially including the two newly erected species *K. berylinis* and *K. wangi* (Wu et al.,

2016), are currently distributed on Taiwan and the Ryukyu Islands, and are limited to these areas. Logically, this group should have originated from nearby mainland China. Many such examples of faunal exchange between the Asian mainland (China) and Taiwan have been previously inferred (Shih et al., 2007; Yuan et al., 2004; Zou et al., 2007). However, lineages B and C (see Fig. 2) were estimated in our analyses to be more closely related to species distributed in Indochina (*K. motokawai*, *K. banaensis*, *K. viridescens*; see Fig. 2) than the other species from South China. This unusual finding may indicate that a southern colonization route is more appropriate than eastern routes previously implied in other studies. Reconstructions of tectonic activity around the South China Sea suggest drastic changes since the opening of the South China Sea at approximately 30 Ma (Mid Oligocene; extending to around 15 Ma; Mid Miocene; Hall, 1998). In addition, the divergence-time estimate indicates that the Taiwanese species originated at 32.8 Ma (5–95% CI: 39.9–25.6 Ma), and this period coincided with the continuously enlarging South China Sea Basin. The temporal coincidence

between the opening of the South China Sea and the origin of the lineage leading to the species from Taiwan supports the south-north coastline-colonization hypothesis as possibly having contributed to initial diversification of *Kurixalus*. On the other hand, despite existing land connections between China and Taiwan island at times of low sea level at various times within the history of *Kurixalus*, any evidence for a direct faunal exchange between China and Taiwan within *Kurixalus* is lacking from the present phylogenetic relationships. A possible explanation could be that the respective autochthonous species blocked the expansion of their congeners across the contact zone between China and Taiwan, and/or areas emergent during times of low sea level were unsuitable for tree-living anurans.

A previous study asserted that speciation between *K. eiffingeri* and *K. idiotocus* (recognized as the genus *Chinrixalus* species at that time) may have been the result of allopatric differentiation between Taiwan and the southern Ryukyus, and that the latter secondarily dispersed into Taiwan (Ota, 1998). However, results of our phylogenetic analyses (see Fig. 3) favour the hypothesis that *K. eiffingeri* first arose in Taiwan, and then, subsequently dispersed into the southern Ryukyu Islands. In accordance with this hypothesis, a terrestrial corridor from Taiwan to the Ryukyus inferred in numerous previous studies (Chiang and Schaal, 2006; Huang et al., 2004; Matsui et al., 2005; Ota, 1998; Shih et al., 2007) could have been a probable conduit for faunal exchange.

4.4. Range evolution in continental Asia

The Red River bisects northern Vietnam, forming a north-south biogeographic barrier that is evident in the geographic distribution of both flora and fauna (Orlov et al., 2013; Vuong and Sridith, 2016). Similarly, the Mekong River, the longest in Southeast Asia, forms an east-west biogeographic barrier (Fooden, 1996; Long et al., 1994; Meijaard and Groves, 2005). Historically, the Mekong River was a major tributary of the Red River system prior to the reorganization of drainage lines in Southeastern Tibet (Clark et al., 2004). The Red River probably originated from left-lateral strike-slip motion at 60 Ma (Lee and Lawver, 1995), or maybe later at 30 Ma (Hall, 1998). Given the placement and timing of these events, it is reasonable to postulate that Red River and Mekong River barriers may have hampered dispersal of fauna in the region. Our analyses nonetheless revealed numerous dispersal events that occurred across these barriers within and between the species *K. baliogaster*, *K. banaensis*, *K. bisacculus*, *K. motokawai*, *K. odontotarsus*, *K. verrucosus* and *K. viridescens* (see Supplement Fig. 1). These high rates of dispersal suggest that the two rivers represent weak barriers, which most likely did not influence or cause speciation. We inferred three major movements of populations between Indochina and southern China (Fig. 4b): (1) colonization of southern China from Indochina around 29 Ma (close to the Late Oligocene Warming event; Zachos et al., 2001), (2) movement of a southern Chinese population to Indochina at approximately 19.2 Ma, (congruent with the Mi-1 glaciation; 24.4–21.5 Ma; Zachos et al., 2001), and (3) movement of an Indochinese population northward into China at about 14 Ma (at about the Mid-Miocene Climatic Optimum; Zachos et al., 2001). Thus, despite the many sources of uncertainty associated with inferring causation, we find climate-change interpretations for range evolution and diversification in *Kurixalus* to be the most plausible current working hypothesis.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

YYL, KH, SK, RMB and JTL collected data, performed analyses and drafted the manuscript. JTL conceived the study and wrote the final manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2017.09.019>.

References

- Acton, G.D., 1999. Apparent polar wander of India since the Cretaceous with implications for regional tectonics and true polar wander. *Mem. Geol. Soc. India* 44, 129–175.
- Ali, J.R., Aitchison, J.C., 2006. Positioning Paleogene Eurasia problem: solution for 60–50 Ma and broader tectonic implications. *Earth Planet. Sc. Lett.* 251, 148–155.
- Bain, R.H., Hurley, M.M., 2001. A biogeographic synthesis of the amphibians and reptiles of Indochina. *Bull. Am. Mus. Nat. Hist.* 360, 1–138.
- Bell, R.C., Drewes, R.C., Zamudio, K.R., 2015. Reed frog diversification in the Gulf of Guinea: overseas dispersal, the progression rule, and in situ speciation. *Evolution* 69, 904–915.
- Briggs, J.C., 2003. The biogeographic and tectonic history of India. *J. Biogeogr.* 30, 381–388.
- Che, J., Zhou, W.W., Hu, J.S., Yan, F., Papenfuss, T.J., Wake, D.B., Zhang, Y.P., 2010. Spiny frogs (Paini) illuminate the history of the Himalayan region and Southeast Asia. *Proc. Natl. Acad. Sci. USA* 107, 13765–13770.
- Chiang, T.Y., Schaal, B.A., 2006. Phylogeography of plants in Taiwan and the Ryukyu Archipelago. *Taxon* 55, 31–41.
- Clark, M.K., Schoenbohm, L.M., Royden, L.H., Whipple, K.X., Burchfiel, B.C., Zhang, X., Tang, W., Wang, E., Chen, L., 2004. Surface uplift, tectonics, and erosion of eastern Tibet from large-scale drainage patterns. *Tectonics* 23.
- De Queiroz, A., 2014. *The Monkey's Voyage: How Improbable Journeys Shaped the History of Life*. Perseus Books Group.
- Dinesh, K., Radhakrishnan, C., Gururaja, K., Deuti, K., Bhatta, G., 2013. A Checklist of Amphibia of India with IUCN Red list Status. *Zoological Survey of India*.
- Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* 32, 1792–1797.
- Folie, A., Rana, R.S., Rose, K.D., Sahni, A., Kumar, K., Singh, L., Smith, T., 2013. Early Eocene frogs from Vastan Lignite Mine, Gujarat, India. *Acta Palaeontol. Pol.* 58, 511–524.
- Fooden, J., 1996. Zoogeography of Vietnamese primates. *Int. J. Primatol.* 17, 845–899.
- Frost, D.R., 2016. *Amphibian Species of the World: An Online Reference*. Version 6.0 (10, December 2016).
- Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., De Sa, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C., 2006. The amphibian tree of life. *Bull. Am. Mus. Nat. Hist.* 8–370.
- Grismer, J.L., Ii, J.A.S., Alexander, A., Wagner, P., Travers, S.L., Buehler, M.D., Welton, L.J., Brown, R.M., 2016. The Eurasian invasion: phylogenomic data reveal multiple Southeast Asian origins for Indian Dragon Lizards. *BMC Evol. Biol.* 16, 1–11.
- Hall, R., 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Robert Hall R., Holloway J.D. (Eds.), *Biogeography and Geological Evolution of SE Asia*, pp. 99–131.
- Hertwig, S.T., Schweizer, M., Das, I., Haas, A., 2013. Diversification in a biodiversity hotspot – the evolution of Southeast Asian rhacophorid tree frogs on Borneo (Amphibia: Anura: Rhacophoridae). *Mol. Phylogenet. Evol.* 68, 567–581.
- Hopkins, G.R., Brodie Jr., E.D., 2015. Occurrence of amphibians in saline habitats: a review and evolutionary perspective. *Herpetol. Monogr.* 29, 1–27.
- Huang, S.F., Hwang, S.Y., Wang, J.C., Lin, T.P., 2004. Phylogeography of *Trochodendron aralioides* (Trochodendraceae) in Taiwan and its adjacent areas. *J. Biogeogr.* 31, 1251–1259.
- Karanth, K.P., 2006. Out-of-India Gondwanan origin of some tropical Asian biota. *Curr. Sci. India* 90, 789–792.
- Katoh, K., Misawa, K., Kuma, K.I., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucl. Acids Res.* 30, 3059–3066.

- Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120.
- Klaus, S., Schubart, C.D., Streit, B., Pfenninger, M., 2010. When Indian crabs were not yet Asian – biogeographic evidence for Eocene proximity of India and Southeast Asia. *BMC Evol. Biol.* 10, 1–9.
- Klaus, S., Morley, R.J., Plath, M., Zhang, Y.P., Li, J.T., 2016. Biotic interchange between the Indian subcontinent and mainland Asia through time. *Nat. Commun.* 7, 12132.
- Lee, T.Y., Lawver, L.A., 1995. Cenozoic plate reconstruction of Southeast Asia. *Tectonophysics* 251, 85–138.
- Leech, M.L., Singh, S., Jain, A.K., Klemperer, S.L., Manickavasagam, R.M., 2005. The onset of India-Asia continental collision: early, steep subduction required by the timing of UHP metamorphism in the western Himalaya. *Earth Planet. Sci. Lett.* 234, 83–97.
- Li, J.T., Che, J., Bain, R.H., Zhao, E.M., Zhang, Y.P., 2008. Molecular phylogeny of Rhacophoridae (Anura): a framework of taxonomic reassignment of species within the genera *Aquixalus*, *Chiromantis*, *Rhacophorus*, and *Philautus*. *Mol. Phylogenet. Evol.* 48, 302–312.
- Li, J.T., Che, J., Murphy, R.W., Zhao, H., Zhao, E.M., Rao, D.Q., Zhang, Y.P., 2009. New insights to the molecular phylogenetics and generic assessment in the Rhacophoridae (Amphibia: Anura) based on five nuclear and three mitochondrial genes, with comments on the evolution of reproduction. *Mol. Phylogenet. Evol.* 53, 509–522.
- Li, J.T., Li, Y., Klaus, S., Rao, D.Q., Hillis, D.M., Zhang, Y.P., 2013. Diversification of rhacophorid frogs provides evidence for accelerated faunal exchange between India and Eurasia during the Oligocene. *Proc. Natl. Acad. Sci. USA* 110, 3441–3446.
- Li, X.H., Wang, C.S., Luba, J., Hu, X.M., 2006. Age of initiation of the India-Asia collision in the east-central Himalaya: a discussion. *J. Geol.* 114, 637–640.
- Long, Y., Kirkpatrick, C.R., Zhongtai, Xiaolin, 1994. Report on the distribution, population, and ecology of the Yunnan snub-nosed monkey (*Rhinopithecus bieti*). *Primates* 35, 241–250.
- Matsui, M., Shimada, T., Ota, H., Tanaka-Ueno, T., 2005. Multiple invasions of the Ryukyu Archipelago by Oriental frogs of the subgenus *Odorrana* with phylogenetic reassessment of the related subgenera of the genus *Rana*. *Mol. Phylogenet. Evol.* 37, 733–742.
- Matzke, N.J., 2013. BioGeoBEARS: Biogeography with Bayesian (and Likelihood) Evolutionary Analysis in R Scripts. R package, version 0.2.1.
- Matzke, N.J., 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst. Biol.* 63, 951–970.
- Meijaard, E., Groves, C.P., 2005. The geography of mammals and rivers in mainland Southeast Asia. In: Lehman, S.M., Fleagle, J.G. (Eds.), *Primate Biogeography*. Springer, US, pp. 305–329.
- Nguyen, T., Matsui, M., Eto, K., 2014a. A new vryptic tree frog species allied to *Kurixalus banaensis* (Anura: Rhacophoridae) from Vietnam. *Russ. J. Herpetol.* 21, 295–302.
- Nguyen, T.T., Matsui, M., Duc, H.M., 2014b. A new tree frog of the genus *Kurixalus* (Anura: Rhacophoridae) from Vietnam. *Curr. Herpetol.* 33, 101–111.
- Orlov, N.L., Lathrop, A., Murphy, R.W., Ho, T.C., 2013. Frogs of the family Rhacophoridae (Anura: Amphibia) in the Northern Hoang Lien Mountains (Mount Fan Si Pan, Sa Pa District, Lao Cai Province), Vietnam. *Russ. J. Herpetol.* 8, 17–44.
- Ota, H., 1998. Geographic patterns of endemism and speciation in amphibians and reptiles of the Ryukyu Archipelago, Japan, with special reference to their paleogeographical implications. *Res. Popul. Ecol.* 40, 189–204.
- Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290.
- Posada, D., 2008. JModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25, 1253–1256.
- Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57, 4–14.
- Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst. Biol.* 46, 195–203.
- Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Ronquist, F., Teslenko, M., Van, d.M.P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542.
- Rowley, J.J.L., Dau, V.Q., Nguyen, T.T., Cao, T.T., Nguyen, S.V., 2011. A new species of *Gracixalus* (Anura: Rhacophoridae) with a hyperextended vocal repertoire from Vietnam. *Zootaxa* 22–38.
- Schettino, A., Scotese, C.R., 2005. Apparent polar wander paths for the major continents (200 Ma to the present day): a palaeomagnetic reference frame for global plate tectonic reconstructions. *Geophys. J. Int.* 163, 727–759.
- Shih, H.T., Fang, S.H., Ng, P.K., 2007. Phylogeny of the freshwater crab genus *Somanniathelphusa* Bott (Decapoda: Parathelphusidae) from Taiwan and the coastal regions of China, with notes on their biogeography. *Invert. Syst.* 21, 29–37.
- Stamatakis, A., 2006. Stamatakis A. RAxML-VI-HP: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models, v. 7.03. *Bioinformatics* 22, 2688–2690.
- Swofford, D., 2002. PAUP: Phylogenetic Analysis Using Parsimony (*and Other Methods) Sunderland. Sinauer Associates, Sunderland, Massachusetts.
- Van der Meijden, A., Vences, M., Hoegg, S., Boistel, R., Channing, A., Meyer, A., 2007. Nuclear gene phylogeny of narrow-mouthed toads (Family: Microhylidae) and a discussion of competing hypotheses concerning their biogeographical origins. *Mol. Phylogenet. Evol.* 44, 1017–1030.
- Vuong, T.B., Sridith, K., 2016. The phytogeographic note on the orchids flora of Vietnam: a case study from the Hon Ba Nature Reserve, Central Vietnam. *Taiwania* 61, 127–140.
- Wu, S.P., Huang, C.C., Tsai, C.L., Lin, T.E., Jhang, J.J., Wu, S.H., 2016. Systematic revision of the Taiwanese genus *Kurixalus* members with a description of two new endemic species (Anura, Rhacophoridae). *Zookeys* 121–153.
- Xia, X., Xie, Z., 2001. DAMBE: software package for data analysis in molecular biology and evolution. *J. Heredity* 92, 371–373.
- Yan, Y., Harris, A.J., Blair, C., He, X., 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Mol. Phylogenet. Evol.* 87, 46–49.
- Yu, G.H., Zhang, M.W., Yang, J.X., 2010. A species boundary within the Chinese *Kurixalus odontotarsus* species group (Anura: Rhacophoridae): new insights from molecular evidence. *Mol. Phylogenet. Evol.* 56, 942–950.
- Yu, G.H., Zhang, M.W., Yang, J.X., 2013. Molecular evidence for taxonomy of *Rhacophorus appendiculatus* and *Kurixalus* species from northern Vietnam, with comments on systematics of *Kurixalus* and *Gracixalus* (Anura: Rhacophoridae). *Biochem. Syst. Ecol.* 47, 31–37.
- Yuan, Y.M., Song, Y., Geuten, K., Rahelivololona, E., Wohlhauser, S., Fischer, E., Smets, E., Küpfer, P., 2004. Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences. *Taxon* 53 391–391.
- Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292, 686–693.
- Zou, F., Lim, H.C., Marks, B.D., Moyle, R.G., Sheldon, F.H., 2007. Molecular phylogenetic analysis of the Grey-cheeked Fulvetta (*Alcippe morrisonia*) of China and Indochina: a case of remarkable genetic divergence in a “species”. *Mol. Phylogenet. Evol.* 44, 165–174.
- Zug, G.R., Vitt, L., Caldwell, J.P., 2001. *Herpetology: An Introductory Biology of Amphibians and Reptiles*. Academic Press.